OBJECTIONS TO INFORMALITIES IN THE CLAIMS

Claims 3, 7 and 13 have been objected to as containing minor informalities. Applicant respectfully submit that the informalities have been corrected in the newly presented claims. Accordingly, Applicant respectfully requests withdrawal of this objection.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claim 16 is rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Cancellation of claim 16 renders the Examiner's rejection moot with respect to that claim. The newly presented claims are believed to satisfy the statute.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 1-12 and 17-20 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Cancellation of claims 1-12 and 17 –20 renders the Examiner's rejections with respect to those claims moot. The newly presented claims incorporate the Examiner's suggestions and are believed to comply with the statute.

REJECTIONS UNDER 35 U.S.C. § 102

Claim 13 is rejected under 35 U.S.C. 102(b) as being anticipated by Levine *et al.* (1993) (American Journal of Epidemiology 138(10):849-869). Specifically, the Examiner contends that "Levine *et al.* teach a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggregative *E. coli* in a sample, wherein for each subgroup of pathogenic *E. coli* a different probe is included in the set." (Office Action, page 8). Cancellation of claim 13 renders the Examiner's rejection with respect to that

claim moot. Applicant traverses this ground rejection with respect to the newly presented claims for the reasons discussed below.

For a finding of anticipation, no difference must exist between the claimed invention and the reference. Specifically, anticipation requires that each and every element as set forth in the claim be present in a single prior art reference. See Davis v. Loesch, 27 U.S.P.Q. 2d 1440 (Fed. Cir. 1993). Instant claim 33 (which corresponds to canceled claim 13) is directed to *a set of labeled oligonucleotide probes,* wherein the set comprises at least one probe specifically hybridizable to one of the five subgroups of pathogenic *E.coli.* In contrast, Levine utilizes four cloned fragments, not oligonucleotides, to detect four of the subgroups of pathogenic *E.coli.* Exclusion of a single claimed element from the prior art reference is enough to negate anticipation by that reference. See Atlas Powder Co. v. E.I. du Pont De Nemours & Co. 224 U.S.P.Q. 409, 411 (Fed. Cir. 1984).

Levine uses cloned fragments of DNA to detect enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC) and enteroaggravative (EaggEc) (page 854, middle paragraph, last sentence). Specifically, EPEC was detected using a probe for the EPEC adherence factor plasmid (page 854, in the middle of the left column) described by Nataro et al., (1985) J. Inf. Diseases 152(56): 560-565 (Attached as Exhibit A). At page 561, right column, Nataro et al. describes the clone as "a purified 1-kb BamHI-SalI fragment". EHEC was detected using a probe for the 60 megadalton EHEC virulence plasmid (page 854, in the middle of the left column) as described in Levine et al. (1987) J. Inf. Diseases 156:175-182 (attached as Exhibit B). At page 177, left column, second paragraph Levine et al. describes the probe as being generated by digesting a plasmid with HindIII. EIEC was detected using a probe for the 140-megadalton invasiveness plasmid (page 854, in the middle of the left column) as described in Wood et al. (1986) J. Clin. Microbiol 24: 498-500 (Attached as Exhibit C). At the bridging paragraph at pages 498-499, Wood et al. describes the probe as a 2.5 kilobase HindIII fragment of the invasiveness plasmid from an enteroinvasive *E. coli* strain. EaggEc was detected using a probe for the EaggEc plasmid (page 854, in the middle of the left column) that encodes both aggravative adherence and enteroaggravative E.coli heat stable enterotoxin as described in

Baudry et al. (1990) J. Infect. Disease 161:1249-51 (Attached as Exhibit D). For only one subgroup of the five subgroups does Levine et al. use a oligonucleotide probe rather than a cloned fragment. Specifically, an oligonucleotide probe was used for the ST-gene of the *E. coli* subgroup ETEC. Accordingly, as Levine et al. does not use a different labeled oligonucleotide probe for each subgroup, wherein each oligonucleotide probe is capable of specifically hybridizing to a subgroup, Levine et al. does not disclose each aspect of the claimed invention, and fails to anticipate the claimed invention. Reconsideration and withdrawal of the § 102 rejection is respectfully requested.

Moreover, as noted by the Examiner (Office Action, page 9, line 4), Levine et al. does not teach PCR amplification of the genes characteristic of the subgroups of pathogenic *E. coli.* Levine et al utilizes colony hybridization, not Real Time PCR as in, for example, claim 33. Once again, as Levine does not disclose each aspect of the claimed invention, it cannot anticipate the claimed invention.

REJECTIONS UNDER 35 U.S.C. § 103

Claims 1, 2, 9, 10, 17, and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Levine et al. in view of Lang et al. (September 1994) Applied and Environmental Microbiology 3145-3149). Specifically, the Examiner contends that it:

> "..would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine et al. so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang et al. in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes." (Office Action, page 9)

Cancellation of claims 1, 2, 9, 10, 17, and 18 obviated the Examiner's rejection with respect to those claims. Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

Applicant's claimed invention is directed to methods of detecting and differentiating all five subgroups of pathogenic *E. coli* in a biological sample by using a set of oligonucleotide primer pairs, wherein the set comprises at least one oligonucleotide primer pair for each pathogenic *E.coli* subgroup, and wherein the primer pair is capable of specifically hybridizing to that subgroup (*e.g.*, claims 21, 22, 29 , 37 and 38) and to a set of oligonucleotide primer pairs (*e.g.*, claim 30) capable of specifically hybridizing to the subgroups, thereby allowing for detection and differentiation of the subgroups of pathogenic *E. coli.* Levine et al., either alone or in combination with Lang et al. does not teach the instantly claimed subject matter.

The Examiner contends that Levine et al. teaches a method for the detection and differentiation of ETEC, EIEC, EPEC, EHEC, and EaggEC using "...oligonucleotides *(emphasis added)* to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC." (Office action, bridging paragraph, pages 8-9) As discussed herein above, Levine et al. uses mostly cloned fragments, not oligonucleotide primer pairs to screen for the subgroups. Moreover, as noted by the Examiner, Levine et al. does not teach a method that utilizes PCR amplification of a DNA sequence. Absent such teachings, Levine et al. cannot render the claimed invention obvious.

Lang *et al.* relates to the amplification of a toxin gene characteristic for one of the two subgroups (*e.g.*, enterotoxigenic and enterohemorrhagic *E. coli* strains) and not detection and differentiation of all five subgroups of pathogenic *E. coli.* The other subgroups of *E.coli* (e.g., EIEC, EPEC and EaggEC) are also the causative agents in food borne illness. Thus, assaying a biological sample with only two of the subgroups may lead to a result which is a false negative and misdiagnosis of an individual. As Lang et al. relates to the amplification of a toxin gene characteristic for one of the two subgroups and not detection and differentiation of all five subgroups of pathogenic E. coli, Lang et al. cannot render the claimed invention obvious either alone or in combination.

Applicant respectfully submits that a proper analysis under 35 USC § 103 requires a consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be found in the prior art, not applicant's disclosure. In re Vaeck 947 Fed, 2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

The art in fact taught away from using a set of oligonucleotide primer pairs in a PCR method to detect and identify the subgroups of pathogenic *E. coli*. For example, Schmidt et al (1995) Journal of Clinical Microbiology 33(8): 2188-2199 (Exhibit E) teaches that PCR may not be sufficient. Schmidt et al. relates to a method for to detect EaggEC strains. Schmidt et al. states that PCR alone is not sufficient for a positive diagnosis of EaggEC strains (page 705, left column) and that two additional tests are required. In fact, Schmidt et al. teaches that a total of three tests (first a test by PCR, second a test by cell culture or hybridization with a probe and third a test by biochemical testing of positive colonies; page 705, left column) are necessary for an accurate diagnosis. In contrast, the instantly claimed invention provides for the first time oligonucleotide primers and probes for the detection and differentiation of all known *E. coli* strains, including EaggEC strains, by PCR alone.

Likewise, Savarino et al.(1993) PNAS 90:3093-3097 (Exhibit F) also teaches away from using a set of oligonucleotide primer pairs in a PCR method to detect and identify the subgroups of pathogenic *E. coli*. Savarino et al. relates to a PCR method for the amplification of the *astA* open reading frame which codes for EaggEC heat stable enterotoxin 1 (page 3094, left column, 1st paragraph). However, the primer (5' TAG GAT CCT CAG GTC GCG ACT GAC GGC 3') utilized by Savarino et al. is not highly specific for EaggEC. As evidenced by the first three hits in the attached Blast search (Exhibit G), the primer was more homologous to *E. coli* O157:H7. *E. coli* O157:H7 is not an EaggEC but an EHEC strain (instant application, page 2, line 10). As evidenced by Schmidt et al. and Savarino et al. the art taught that PCR could not be utilized to detect and differentiate the subgroups of pathogenic *E.coli*. The skilled artisan therefore would

not have been motivated or had a reasonable expectation of success of utilizing PCR to detect and differentiate the subgroups of pathogenic *E.coli.* The suitability of the PCR to detect and differentiate the subgroups of pathogenic *E.coli* is not described in the art, but only in the instant disclosure. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim 3 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of Lang et al. as applied to claims 1, 2, 9, 10, 17, and 18 above and further in view of Yamamoto et al. (1983) Journal of Bacteriology 155(2): 728-733), Paton et al. (GenBank Z36899) and of Hogan et al. (US 5595874). Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

Levine et al. either alone or in combination with Lang et al. does not render the claimed invention obvious for the reasons provided herein above. Neither Yamamoto et al., Paton et al. or Hogan remedy that deficiency. Yamamoto et al relates to the full length sequence of the heat labile toxin gene of enterotoxigenic *E. coli* strains, but does not teach or suggest the specific primers or probes used to detect the heat labile toxin gene of enterotoxigenic *E. coli* strains claimed in the instant invention. Paton et al. relates to the full length SLT1 gene, but does not teach or suggest the specific primers or probes used to detect the SLT1 gene in the instant application. Hogan relates generally to selection of primers but does not teach or suggest the instantly claimed primers or probes. Yamamoto et al., Paton et al. or Hogan do not teach or suggest the instantly claimed oligonucleotide primers or probes capable of specifically hybridizing and amplifying all five subgroups of pathogenic *E. coli.* Thus, these references either alone or in combination do not render the claimed invention obvious.

Claims 4, 5, 6, and 8 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over as being unpatentable over Levine et al. in view of Lang et al. as applied to claims 1, 2, 9, 10, 17, and 18 above and further in view of in view of Livak et al. (PCR Methods and Applications (1995) 4:357-362). Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

Livak et al. is a general review relating to the use of probes labeled at 5' and 3' end for use in a 5' nuclease PCR assay. Livak et al. neither teaches or suggests labeled oligonucleotide probes that specifically hybridize to a DNA sequence characteristic of a virulence factor or toxin for the five subgroups of pathogenic *E. Coli*. Livak et al. does not remedy the deficiencies of the earlier cited references and , therefore cannot render the claimed invention obvious.

Claim 7 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of Lang et al. in view of Livak et al. as applied to claims 4, 5, 6, and 8 above, and further in view of Yamamoto et al., Paton et al. (GenBank Z36899) and of Hogan et al. Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

New claim 27, which corresponds to canceled claim 7, recites specific oligonucleotide primers capable of specifically hybridizing to the virulence/toxin factors characteristic of the known subgroups of pathogenic *E. coli*. None of the cited references either alone or in combination disclose the set of specific oligonucleotide probes recited in claim 27. Accordingly, these references cannot render the claimed invention obvious and withdrawal of this ground of rejection is respectfully requested.

Claim 11 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of Lang et al., as applied to claims 1, 2, 8, 10, 17 and 18 above, and further in view of Savarino et al. 1993 PNAS (USA) 90: 3093-3097. As discussed herein above, Savarino teaches away from the instantly claimed invention. Accordingly, Savarino does not remedy the deficiencies of Levine et al. or Lang et al. Withdrawal of this ground of rejection is respectfully requested.

Claim 14 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of both Savarino et al. and Louie et al. (1994) Epidemiology. Infect. 112:449-461. Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

The Examiner alleges that Louie et al teaches PCR assays and probes that allow for specific identification pof EPEC genes. Two of the four primer pairs in Louie et al., specifically primer pair C1 and C2 and S1 and S2, do not specifically recognize EPEC. Moreover, Louie et al does not provide apecific primer pairs and probes for all five subgroups of pathogenic *E. coli*. Such a disclosure is found only in the instant application. Accordingly, Louie et al., either alone or in combination, does not render the claimed invention obvious. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim 15 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of both Savarino et al. and Louie et al. as applied to claim 14 above, and further in view of all of the following: Yamamoto et al., Moseley et al. (GenBank M34916), Yamamoto et al. (1996) Infection and Immunology 64 (4):1441-1445, Schmidt *et al.* (1995) Journal of Clinical Microbiology 33(3): 701-705), Lampel et al. (US 5041372), Franke et al. (1994) Journal of Clinical Microbiology (1994) 32(10):2460-2463, Kaper (1995), GenBank Accession Z11541, Paton *et al.* (GenBank Z36899), and Paton *et al.* (GenBank L111079). Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

New claim 25, which corresponds to canceled claim 15, recites specific oligonucleotide primers capable of specifically hybridizing to the virulence/toxin factors characteristic of the known subgroups of pathogenic *E. coli.* None of the cited references either alone or in combinations teach or suggest the specific set of primers recited in claim 25. Accordingly, these references cannot render the claimed invention obvious and withdrawal of this ground of rejection is respectfully requested.

Claims 16 is rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of Lang et al. and further in view of the Stratagene Catalog. As argued above Levine et al in combination with Lang et al does not render the claimed invention obvious. The Stratagene Catalog does not remedy this deficiency. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claims 17, 19, and 20 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Levine et al. in view of Lang et al. in view of Tsen et al. (1996) Journal of Food Protection 59(8): 795-802 (ABSTRACT ONLY). Applicant traverses this rejection with respect to the newly presented claims for the reasons presented below.

As argued above Levine et al in combination with Lang et al does not render the claimed invention obvious either alone or in combination. Tsen does not remedy this deficiency as it does not teach or suggest detection and differentiation via PCR of all the subgroups of pathogenic *E. coli* Accordingly, withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims comply with 35 U.S.C. § 112, first and second paragraph and define an invention that is patentable over the art. Accordingly, allowance is in order, and an early notification to that effect would be appreciated. Should the Examiner in reviewing the communication have any questions or need any additional information, she is welcome to contact the undersigned at (650) 849-4902.

This Amendment is in response to the Office Action dated August 29, 2001. the Office Action Summary Sheet listed this Office Action as Final. However, in the text of the Office Action (page 2, first paragraph) the term Final was crossed out. Applicant confirmed with Examiner Einsmann that the Office Action was non-final. However, in the event that this Office Action is a Final Office Action, Applicant hereby appeals to the Board of Appeals from the decision dated August 29, 2001 of the Examiner rejecting claims 1-20. Please charge Deposit Account No. 50-1189, Docket No. 20239-706 in the amount of $320.00 to cover the Notice of Appeal Fee. Thw Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1189, Docket No. 20239-706.

Docket No.: 20239-706

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required by this paper, or credit any overpayment to Deposit Account No. 50-1189. Docket No.: 20239-706. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

Dated: February 28, 2002

By: _____

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Docket No.: 20239-706

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